Syntheses, characterisation and photophysical studies of novel biological labelling reagents derived from luminescent iridium(III) terpyridine complexes

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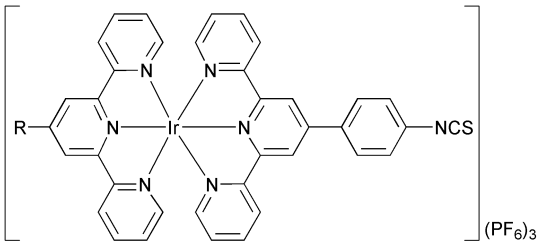
A series of new luminescent iridium(III) terpyridine complexes functionalised with an isothiocyanate group, [Ir(tpy-R)(tpy-C_6H_4-NCS-*p*)]$(PF_6)_3$ [R = H (**1**), C_6H_5 (**2**), C_6H_4-CH_3-*p* (**3**), C_6H_4-Cl-*p* (**4**)] has been synthesised, characterised and their photophysical properties studied; the X-ray crystal structure of one of the intermediate complexes, [Ir(tpy-C_6H_4-Cl-*p*)$(CF_3SO_3)_3$] (**4b**), has also been determined; complex **1** has been used as a luminescent label for proteins.

The design of transition metal complexes that can bind and/or react at specific locations of biomolecules has been arousing much interest.[1] By virtue of their flexible coordination geometry and rich photophysical and electrochemical properties, many transition metal complexes have been covalently linked to nucleoside phosphoramidites for solid-phase DNA synthesis, as well as other biological molecules such as nucleic acids, peptides and proteins for a wide range of mechanistic and analytical investigations.[2–18] While the design of many luminescent biological probes has relied on ruthenium(II),[3a–c,6a,7,8a,b,9–12,15–18] and recently osmium(II)[6d,8d,17] and rhenium(I)[3b,d,8c,e] complexes, the possibility of using the isoelectronic iridium(III) complexes as luminescent biological labelling reagents has not been explored.

The photoluminescence properties of iridium(III) complexes have been known for many years.[19–27] Their photophysical properties have also been shown to provide unique advantages over their d^6 counterparts. In terms of molecular structures, iridium(III) shows a higher variety, including its remarkable ability to form mono-, bis- and triscyclometallated complexes. The high structural variety allows better control of the excited-state nature and emission properties of these complexes. With different polypyridine ligands[19,21,25,27] and/or cyclometallating ligands,[20,22–24,26] luminescent iridium(III) complexes can offer a wider range of emission energy and, in many cases, longer emission lifetimes compared to the ruthenium(II) analogues.

Recently, Williams and co-workers described the utilisation of a series of interesting luminescent iridium(III) terpyridine complexes as pH[27a] and chloride ion[27b] probes. With this in mind, we believe that luminescent iridium(III) polypyridine complexes are promising candidates for various bioanalytical applications. It is also anticipated that these complexes can offer additional advantages over traditional organic fluorophores[28] in biological labelling in consideration of their long-lived and intense luminescence, large Stokes' shifts and high photostability. In this paper, we report the syntheses, characterisation and photophysical properties of a series of new luminescent iridium(III) terpyridine complexes functionalised with an isothiocyanate moiety, [Ir(tpy-R)(tpy-C_6H_4-NCS-*p*)]$(PF_6)_3$ [tpy-R = 4′-substituted 2,2′:6′,2″-terpyridine, where R = H (**1**), C_6H_5 (**2**), C_6H_4-CH_3-*p* (**3**), C_6H_4-Cl-*p* (**4**);



R = -H (**1**), -C_6H_5 (**2**), -C_6H_4-CH_3-*p* (**3**), -C_6H_4-Cl-*p* (**4**)

Scheme 1

tpy-C_6H_4-NCS-*p* = 4′-(4-isothiocyanatophenyl)-2,2′:6′,2″-terpyridine] (Scheme 1). The incorporation of the isothiocyanate group allows these complexes to react with the primary amine groups of biological substrates to form bioconjugates with stable thiourea linkages.[28,29] On the other hand, the syntheses of **2**–**4** have involved the use of a series of new precursor complexes, [Ir(tpy-R)$(CF_3SO_3)_3$] [R = C_6H_5 (**2b**), C_6H_4-CH_3-*p* (**3b**), C_6H_4-Cl-*p* (**4b**)]. The X-ray crystal structure of one of these intermediates, **4b**, has also been studied.

Experimental

Materials and reagents

All solvents were of analytical reagent grade. $IrCl_3 \cdot 3H_2O$, 2,2′:6′,2″-terpyridine and thiophosgene were purchased from Aldrich and were used without purification. Human serum albumin (HSA) fraction V and bovine serum albumin (BSA) were obtained from Calbiochem and were used as received. All buffer components were of molecular biology grade and used without purification. The 4′-aryl-substituted 2,2′:6′,2″-terpyridine derivatives tpy-R (R = C_6H_5, C_6H_4-CH_3-*p*, C_6H_4-Cl-*p*, C_6H_4-NO_2-*p*) were synthesised from the reactions of 2-acetylpyridine, ammonium acetate, acetamide and the corresponding benzaldehydes according to reported procedures.[30] The ligand

tpy-C_6H_4-NH_2-*p* was prepared from the reduction of tpy-C_6H_4-NO_2-*p* by hydrazine monohydrate and palladium on charcoal in refluxing ethanol based on a related synthesis.[31] The trichloroiridium(III) terpyridine complexes [Ir(tpy-R)Cl_3] (R = H, C_6H_5, C_6H_4-CH_3-*p*, C_6H_4-Cl-*p*) were synthesised from the reactions of $IrCl_3 \cdot 3H_2O$ and the corresponding terpyridines in degassed ethylene glycol at 160 °C for 20 min.[19b,27]

Syntheses

[Ir(tpy-H)(tpy-C_6H_4-NH_2-*p*)]$(PF_6)_3$ (1a). A mixture of [Ir(tpy-H)Cl_3] (90 mg, 0.17 mmol) and tpy-C_6H_4-NH_2-*p* (55 mg, 0.17 mmol) in degassed ethylene glycol (10 ml) was heated at 160 °C for 20 min under an inert atmosphere of nitrogen in the dark. The mixture was then cooled to room temperature and a saturated aqueous solution of NH_4PF_6 was added to precipitate an orange-red solid. The solid was washed with cold water and then a mixture of methanol and ether, and then dried *in vacuo*. Subsequent recrystallisation of the complex from acetone–diethyl ether afforded **1a** as air-stable orange-red crystals. Yield: 100 mg (50%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, relative to TMS): δ 9.44 (s, 2H, H3′ and H5′ of tpy-C_6H_4-NH_2-*p*), 9.27 (d, 2H, J = 8.5 Hz, H6 and H6″ of tpy-H), 9.17 (d, 2H, J = 7.3 Hz, H6 and H6″ of tpy-C_6H_4-NH_2-*p*), 9.00 (t, 1H, J = 7.9 Hz, H4′ of tpy-H), 8.99 (d, 2H, J = 6.7 Hz, H3′ and H5′ of tpy-H), 8.43–8.37 (m, 4H, H4 and H4″ of tpy-H, H4 and H4″ of tpy-C_6H_4-NH_2-*p*), 8.33 (d, 2H, J = 5.9 Hz, H3 and H3″ of tpy-H), 8.22 (d, 2H, J = 8.8 Hz, H_o of tpy-C_6H_4-NH_2-*p*), 8.16 (d, 2H, J = 5.6 Hz, H3 and H3″ of tpy-C_6H_4-NH_2-*p*), 7.70–7.60 (m, 4H, H5 and H5″ of tpy-H, H5 and H5″ of tpy-C_6H_4-NH_2-*p*), 7.02 (d, 2H, J = 8.8 Hz, H_m of tpy-C_6H_4-NH_2-*p*), 5.84 (s, 2H, NH_2). Positive-ion ESI-MS: m/z 374 $\{[Ir(tpy\text{-}H)(tpy\text{-}C_6H_4\text{-}NH_2\text{-}p)]^{3+} + e^-\}^{2+}$. IR (KBr) ν/cm^{-1}: 838 (s, PF_6^-). Anal. calcd for $C_{36}H_{27}N_7P_3F_{18}Ir$: C, 36.50; H, 2.30; N, 8.28; found C, 36.61; H, 2.37; N, 8.25.

[Ir(tpy-C_6H_5)$(CF_3SO_3)_3$] (2b). A mixture of [Ir(tpy-C_6H_5)Cl_3] (290 mg, 0.48 mmol) and trifluoromethanesulfonic acid (2.1 ml, 23.65 mmol) was refluxed in 1,2-dichlorobenzene (25 ml) under an inert atmosphere of nitrogen in the dark for 12 h. The mixture was then cooled to room temperature. The solvent and the excess acid were carefully removed by decantation. The brownish yellow semi-solid left was washed with a copious amount of petroleum ether and then dissolved in CH_2Cl_2 (5 ml) and loaded onto a chromatographic column. Alumina was used as the stationary phase and CH_2Cl_2 as the eluent. The first yellow band was collected and evaporated to dryness. Subsequent recrystallisation from acetone–petroleum ether afforded [Ir(tpy-C_6H_5)$(CF_3SO_3)_3$] as orange-yellow crystals. Yield: 360 mg (80%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, relative to TMS): δ 9.33 (d, 2H, J = 5.6 Hz, H6 and H6″), 9.26 (s, 2H, H3′ and H5′), 9.07 (d, 2H, J = 7.9 Hz, H3 and H3″), 8.57 (dt, 2H, J = 7.9 and 1.5 Hz, H4 and H4″), 8.33–8.27 (m, 4H, H_o, H5 and H5′), 7.74 (t, 2H, J = 7.0 Hz, H_m), 7.68–7.63 (m, 1H, H_p). Positive-ion ESI-MS: m/z 799 $\{[Ir(tpy\text{-}C_6H_5)(CF_3SO_3)_2]\}^+$. IR (KBr) ν/cm^{-1}: 1348 (s, CF_3SO_3), 1237 (s, CF_3SO_3), 1197 (s, CF_3SO_3), 1019 (s, CF_3SO_3), 974 (s, CF_3SO_3). Anal. calcd for $C_{24}H_{15}N_3F_9S_3O_9Ir$: C, 30.38; H, 1.59; N, 4.43; found C, 30.51; H, 1.50; N, 4.13.

[Ir(tpy-C_6H_4-CH_3-*p*)$(CF_3SO_3)_3$] (3b). The synthesis was similar to that for **2b** except that [Ir(tpy-C_6H_4-CH_3-*p*)Cl_3] (297 mg, 0.48 mmol) was used instead of [Ir(tpy-C_6H_5)Cl_3]. Complex **3b** was isolated as orange-yellow crystals. Yield: 290 mg (63%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, relative to TMS): δ 9.33 (dd, 2H, J = 5.5 and 1.1 Hz, H6 and H6″), 9.23 (s, 2H, H3′ and H5′), 9.06 (d, 2H, J = 7.7 Hz, H3 and H3″), 8.56 (dt, 2H, J = 5.8 and 1.4 Hz, H4 and H4″), 8.29 (ddd, 2H, J = 7.7, 5.8 and 1.4 Hz, H5 and H5″), 8.20 (d, 2H, J = 8.2 Hz, H_o), 7.56 (d, 2H, J = 8.0 Hz, H_m), 2.54 (s, 3H, CH_3). Positive-ion ESI-MS: m/z 813 $\{[Ir(tpy\text{-}C_6H_4\text{-}CH_3\text{-}p)(CF_3SO_3)_2]\}^+$. IR (KBr) ν/cm^{-1}: 1340 (s, CF_3SO_3), 1252 (s, CF_3SO_3), 1196 (s, CF_3SO_3), 1019 (m, CF_3SO_3), 975 (s, CF_3SO_3). Anal. calcd for $C_{25}H_{17}N_3F_9S_3O_9Ir$: C, 31.19; H, 1.78; N, 4.36; found C, 31.01; H, 1.50; N, 4.14.

[Ir(tpy-C_6H_4-Cl-*p*)$(CF_3SO_3)_3$] (4b). The synthesis was similar to that for **2b** except that [Ir(tpy-C_6H_4-Cl-*p*)Cl_3] (306 mg, 0.48 mmol) was used instead of [Ir(tpy-C_6H_5)Cl_3]. Complex **4b** was isolated as orange-yellow crystals. Yield: 358 mg (76%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, relative to TMS): δ 9.33 (d, 2H, J = 6.1 Hz, H6 and H6″), 9.29 (s, 2H, H3′ and H5′), 9.05 (d, 2H, J = 7.4 Hz, H3 and H3″), 8.57 (dt, 2H, J = 8.0 and 1.4 Hz, H4 and H4″), 8.35–8.28 (m, 4H, H_o, H5 and H5″), 7.78 (d, 2H, J = 8.5 Hz, H_m). Positive-ion ESI-MS: m/z 833 $\{[Ir(tpy\text{-}C_6H_4\text{-}Cl\text{-}p)(CF_3SO_3)_2]\}^+$. IR (KBr) ν/cm^{-1}: 1347 (s, CF_3SO_3), 1233 (s, CF_3SO_3), 1202 (s, CF_3SO_3), 1018 (m, CF_3SO_3), 974 (s, CF_3SO_3). Anal. calcd for $C_{24}H_{14}N_3F_9S_3O_9ClIr$: C, 29.32; H, 1.44; N, 4.27; found C, 29.36; H, 1.38; N, 4.09.

[Ir(tpy-C_6H_5)(tpy-C_6H_4-NH_2-*p*)]$(PF_6)_3$ (2a). A mixture of **2b** (180 mg, 0.19 mmol) and tpy-C_6H_4-NH_2-*p* (62 mg, 0.19 mmol) in degassed ethylene glycol (10 ml) was heated at 160 °C for 20 min under an inert atmosphere of nitrogen in the dark. The mixture was then cooled to room temperature and a saturated aqueous solution of NH_4PF_6 was added to precipitate an orange-red solid. The solid was washed with cold water and then a mixture of methanol and ether, and then dried *in vacuo*. The solid was then purified by column chromatography (silica gel) using gradient elution from CH_3CN to CH_3CN–H_2O–saturated aqueous KNO_3 (70 : 27.5 : 2.5). The product was then converted to the PF_6^- salt by metathesis. Subsequent recrystallisation of the complex from acetone–diethyl ether afforded **2a** as air-stable orange-red crystals. Yield: 122 mg (51%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, relative to TMS): δ 9.47 (s, 2H, H3′ and H5′ of tpy-C_6H_5), 9.33 (s, 2H, H3′ and H5′ of tpy-C_6H_4-NH_2-*p*), 9.11 (d, 2H, J = 8.8 Hz, H6 and H6″ of tpy-C_6H_5), 9.08 (d, 2H, J = 9.1 Hz, H6 and H6″ of tpy-C_6H_4-NH_2-*p*), 8.36–8.28 (m, 6H, H4, H4″ and H_o of tpy-C_6H_5, H4 and H4″ of tpy-C_6H_4-NH_2-*p*), 8.25 (d, 2H, J = 4.7 Hz, H3 and H3″ of tpy-C_6H_5), 8.22 (d, 2H, J = 8.8 Hz, H_o of tpy-C_6H_4-NH_2-*p*), 8.15 (d, 2H, J = 5.0 Hz, H3 and H3″ of tpy-C_6H_4-NH_2-*p*), 7.79–7.73 (m, 3H, H_m and H_p of tpy-C_6H_5), 7.64–7.54 (m, 4H, H5 and H5″ of tpy-C_6H_5, H5 and H5″ of tpy-C_6H_4-NH_2-*p*), 7.00 (d, 2H, J = 8.8 Hz, H_m of tpy-C_6H_4-NH_2-*p*), 5.65 (s, 2H, NH_2). Positive-ion ESI-MS: m/z 485 $\{[Ir(tpy\text{-}C_6H_5)(tpy\text{-}C_6H_4\text{-}NH_2\text{-}p)](PF_6)\}^{2+}$. IR (KBr) ν/cm^{-1}: 840 (s, PF_6^-). Anal. calcd for $C_{42}H_{31}N_7P_3F_{18}Ir$: C, 40.01; H, 2.48; N, 7.78; found C, 40.15; H, 2.54; N, 8.04.

[Ir(tpy-C_6H_4-CH_3-*p*)(tpy-C_6H_4-NH_2-*p*)]$(PF_6)_3$ (3a). The synthesis was similar to that for **2a** except that **3b** (183 mg, 0.19 mmol) was used instead of **2b**. Complex **3a** was isolated as orange-red crystals. Yield: 99 mg (41%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, relative to TMS): δ 9.47 (s, 2H, H3′ and H5′ of tpy-C_6H_4-CH_3-*p*), 9.34 (s, 2H, H3′ and H5′ of tpy-C_6H_4-NH_2-*p*), 9.12 (d, 2H, J = 7.9 Hz, H6 and H6″ of tpy-C_6H_4-CH_3-*p*), 9.09 (d, 2H, J = 8.5 Hz, H6 and H6″ of tpy-C_6H_4-NH_2-*p*), 8.36–8.25 (m, 8H, H4, H4″, H3, H3″ and H_o of tpy-C_6H_4-CH_3-*p*, H4 and H4″ of tpy-C_6H_4-NH_2-*p*), 8.22 (d, 2H, J = 8.8 Hz, H_o of tpy-C_6H_4-NH_2-*p*), 8.17 (d, 2H, J = 5.3 Hz, H3 and H3″ of tpy-C_6H_4-NH_2-*p*), 7.64–7.55 (m, 6H, H5, H5″ and H_m of tpy-C_6H_4-CH_3-*p*, H5 and H5″ of tpy-C_6H_4-NH_2-*p*), 7.00 (d, 2H, J = 8.8 Hz, H_m of tpy-C_6H_4-NH_2-*p*), 5.68 (s, 2H, NH_2), 2.55 (s, 3H, CH_3). Positive-ion ESI-MS: m/z 492 $\{[Ir(tpy\text{-}C_6H_4\text{-}CH_3\text{-}p)(tpy\text{-}C_6H_4\text{-}NH_2\text{-}p)](PF_6)\}^{2+}$. IR (KBr) ν/cm^{-1}: 840 (s,

PF_6^-). Anal. calcd for $C_{43}H_{33}N_7P_3F_{18}Ir$: C, 40.51; H, 2.61; N, 7.69; found C, 40.49; H, 2.40; N, 7.54.

[Ir(tpy-C_6H_4-Cl-*p*)(tpy-C_6H_4-NH_2-*p*)]$(PF_6)_3$ (4a). The synthesis was similar to that for **2a** except that **4b** (187 mg, 0.19 mmol) was used instead of **2b**. Complex **4a** was isolated as orange-red crystals. Yield: 115 mg (47%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, relative to TMS): δ 9.45 (s, 2H, H3′ and H5′ of tpy-C_6H_4-Cl-*p*), 9.31 (s, 2H, H3′ and H5′ of tpy-C_6H_4-NH_2-*p*), 9.07 (d, 4H, $J = 7.9$ Hz, H6 and H6″ of tpy-C_6H_4-Cl-*p*, H6 and H6″ of tpy-C_6H_4-NH_2-*p*), 8.37–8.27 (m, 6H, H4, H4″ and H_o of tpy-C_6H_4-Cl-*p*, H4 and H4″ of tpy-C_6H_4-NH_2-*p*), 8.23 (d, 2H, $J = 4.7$ Hz, H3 and H3″ of tpy-C_6H_4-Cl-*p*), 8.21 (d, 2H, $J = 8.2$ Hz, H_o of tpy-C_6H_4-NH_2-*p*), 8.11 (d, 2H, 5.0 Hz, H3 and H3″ of tpy-C_6H_4-NH_2-*p*), 7.78 (d, 2H, $J = 8.8$ Hz, H_m of tpy-C_6H_4-Cl-*p*), 7.64–7.53 (m, 4H, H5 and H5″ of tpy-C_6H_4-Cl-*p*, H5 and H5″ of tpy-C_6H_4-NH_2-*p*), 7.00 (d, 2H, $J = 8.5$ Hz, H_m of tpy-C_6H_4-NH_2-*p*), 5.61 (s, 2H, NH_2). Positive-ion ESI-MS: m/z 429 $\{[Ir(tpy\text{-}C_6H_4\text{-}Cl\text{-}p)(tpy\text{-}C_6H_4\text{-}NH_2\text{-}p)]^{3+} + e^-\}^{2+}$. IR (KBr) ν/cm^{-1}: 841 (s, PF_6^-). Anal. calcd for $C_{42}H_{30}N_7P_3F_{18}ClIr$: C, 38.95; H, 2.33; N, 7.57; found C, 38.87; H, 2.40; N, 7.67.

[Ir(tpy-H)(tpy-C_6H_4-NCS-*p*)]$(PF_6)_3$ (1). To a mixture of **1a** (100 mg, 84.4 μmol) and $CaCO_3$ (34 mg, 339.7 μmol) in 8 ml of dry acetone was added $CSCl_2$ (13 μl, 170.5 μmol). After being stirred for 2 h in the dark under nitrogen, the suspension was filtered and evaporated to dryness to yield an orange-yellow solid. Recrystallisation from acetone–petroleum ether afforded **1** as orange-yellow crystals. Yield: 60 mg (58%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, relative to TMS): δ 9.64 (s, 2H, H3′ and H5′ of tpy-C_6H_4-NCS-*p*), 9.28 (d, 2H, $J = 8.2$ Hz, H6 and H6″ of tpy-H), 9.19 (d, 2H, $J = 8.0$ Hz, H6 and H6″ of tpy-C_6H_4-NCS-*p*), 9.00 (t, 1H, $J = 9.3$ Hz, H4′ of tpy-H), 8.99 (d, 2H, $J = 8.2$ Hz, H3′ and H5′ of tpy-H), 8.45–8.37 (m, 6H, H_o, H4 and H4″ of tpy-C_6H_4-NCS-*p*, H4 and H4″ of tpy-H), 8.25 (d, 2H, $J = 4.7$ Hz, H3 and H3″ of tpy-H), 8.19 (d, 2H, $J = 5.2$ Hz, H3 and H3″ of tpy-C_6H_4-NCS-*p*), 7.80 (d, 2H, $J = 8.5$ Hz, H_m of tpy-C_6H_4-NCS-*p*), 7.65 (br s, 4H, H5 and H5″ of tpy-H, H5 and H5″ of tpy-C_6H_4-NCS-*p*). Positive-ion ESI-MS: m/z 468 $\{[Ir(tpy\text{-}H)(tpy\text{-}C_6H_4\text{-}NCS\text{-}p)](PF_6)\}^{2+}$. IR (KBr) ν/cm^{-1}: 2095 (m, NCS), 840 (s, PF_6^-). Anal. calcd for $C_{37}H_{25}N_7P_3F_{18}SIr$: C, 36.22; H, 2.05; N, 7.99; found C, 36.23; H, 2.10; N, 7.86.

[Ir(tpy-C_6H_5)(tpy-C_6H_4-NCS-*p*)]$(PF_6)_3$ (2). The synthesis was similar to that for **1** except that **2a** (106 mg, 84.4 μmol) was used instead of **1a**. Complex **2** was isolated as yellow crystals. Yield: 65 mg (59%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, relative to TMS): δ 9.58 (s, 2H, H3′ and H5′ of tpy-C_6H_4-NCS-*p*), 9.56 (s, 2H, H3′ and H5′ of tpy-C_6H_5), 9.19–9.14 (m, 4H, H6 and H6″ of tpy-C_6H_4-NCS-*p*, H6 and H6″ of tpy-C_6H_5), 8.45–8.32 (m, 8H, H_o, H4 and H4″ of tpy-C_6H_4-NCS-*p*, H_o, H4 and H4″ of tpy-C_6H_5), 8.25–8.21 (m, 4H, H3 and H3″ of tpy-C_6H_4-NCS-*p*, H3 and H3″ of tpy-C_6H_5), 7.81–7.74 (m, 5H, H_m of tpy-C_6H_4-NCS-*p*, H_m and H_p of tpy-C_6H_5), 7.66–7.60 (m, 4H, H5 and H5″ of tpy-C_6H_4-NCS-*p*, H5 and H5″ of tpy-C_6H_5). Positive-ion ESI-MS: m/z 506 $\{[Ir(tpy\text{-}C_6H_5)(tpy\text{-}C_6H_4\text{-}NCS\text{-}p)](PF_6)\}^{2+}$. IR (KBr) ν/cm^{-1}: 2089 (w, NCS), 841 (s, PF_6^-). Anal. calcd for $C_{43}H_{29}N_7P_3F_{18}SIr$: C, 39.64; H, 2.24; N, 7.53; found C, 39.69; H, 2.00; N, 7.49.

[Ir(tpy-C_6H_4-CH_3-*p*)(tpy-C_6H_4-NCS-*p*)]$(PF_6)_3$ (3). The synthesis was similar to that for **1** except that **3a** (108 mg, 84.4 μmmol) was used instead of **1a**. Complex **3** was isolated as yellow crystals. Yield: 81 mg (73%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, relative to TMS): δ 9.58 (s, 2H, H3′ and H5′ of tpy-C_6H_4-NCS-*p*), 9.54 (s, 2H, H3′ and H5′ of tpy-C_6H_4-CH_3-*p*), 9.18–9.15 (m, 4H, H6 and H6″ of tpy-C_6H_4-NCS-*p*, H6 and H6″ of tpy-C_6H_4-CH_3-*p*), 8.43 (d, 2H, $J = 8.8$ Hz, H_o of tpy-C_6H_4-NCS-*p*), 8.42–8.36 (m, 4H, H4 and H4″ of tpy-C_6H_4-NCS-*p*, H4 and H4″ of tpy-C_6H_4-CH_3-*p*), 8.28–8.21 (m, 6H, H_o, H3 and H3″ of tpy-C_6H_4-CH_3-*p*, H3 and H3″ of tpy-C_6H_4-NCS-*p*), 7.78 (d, 2H, $J = 8.2$ Hz, H_m of tpy-C_6H_4-NCS-*p*), 7.66–7.59 (m, 6H, H_m, H5 and H5″ of tpy-C_6H_4-CH_3-*p*, H5 and H5″ of tpy-C_6H_4-NCS-*p*), 2.55 (m, 3H, CH_3). Positive-ion ESI-MS: m/z 513 $\{[Ir(tpy\text{-}C_6H_4\text{-}CH_3\text{-}p)(tpy\text{-}C_6H_4\text{-}NCS\text{-}p)](PF_6)\}^{2+}$. IR (KBr) ν/cm^{-1}: 2093 (m, NCS), 838 (s, PF_6^-). Anal. calcd for $C_{44}H_{31}N_7P_3F_{18}SIr$: C, 40.13; H, 2.37; N, 7.45; found C, 39.95; H, 2.29; N, 7.31.

[Ir(tpy-C_6H_4-Cl-*p*)(tpy-C_6H_4-NCS-*p*)]$(PF_6)_3$ (4). The synthesis was similar to that for **1** except that **4a** (109 mg, 84.4 μmol) was used instead of **1a**. Complex **4** was isolated as yellow crystals. Yield: 45 mg (40%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, relative to TMS): δ 9.58 (s, 2H, H3′ and H5′ of tpy-C_6H_4-NCS-*p*), 9.57 (s, 2H, H3′ and H5′ of tpy-C_6H_4-Cl-*p*), 9.17–9.14 (m, 4H, H6 and H6″ of tpy-C_6H_4-NCS-*p*, H6 and H6″ of tpy-C_6H_4-Cl-*p*), 8.42–8.35 (m, 8H, H_o, H4 and H4″ of tpy-C_6H_4-NCS-*p*, H_o, H4 and H4″ of tpy-C_6H_4-Cl-*p*), 8.24–8.22 (m, 4H, H3 and H3″ of tpy-C_6H_4-NCS-*p*, H3 and H3″ of tpy-C_6H_4-Cl-*p*), 7.82 (d, 2H, $J = 8.21$ Hz, H_m of tpy-C_6H_4-NCS-*p*), 7.78 (d, 2H, $J = 8.80$ Hz, H_m of tpy-C_6H_4-Cl-*p*), 7.66–7.60 (m, 4H, H5 and H5″ of tpy-C_6H_4-NCS-*p*, H5 and H5″ of tpy-C_6H_4-Cl-*p*). Positive-ion ESI-MS: m/z 523 $\{[Ir(tpy\text{-}C_6H_4\text{-}Cl\text{-}p)(tpy\text{-}C_6H_4\text{-}NCS\text{-}p)](PF_6)\}^{2+}$. IR (KBr) ν/cm^{-1}: 2089 (w, NCS), 842 (s, PF_6^-). Anal. calcd for $C_{43}H_{28}N_7P_3F_{18}SClIr$: C, 38.62; H, 2.11; N, 7.33; found C, 38.69; H, 2.39; N, 7.27.

Labelling of HSA and BSA with complex 1

In a typical labelling reaction, complex **1** (3.0 mg, 2.45 μmol) in 20 μl anhydrous DMSO was added to HSA (3.0 mg, 45.5 nmol) or BSA (3.0 mg, 45.5 nmol) dissolved in 180 μl of 50 mM carbonate buffer pH 9.1. The suspension was stirred slowly in the dark at room temperature for 48 h. The solid residue was then removed by centrifugation. The supernatant was diluted to 1.0 ml with 50 mM Tris-HCl pH 7.4 and loaded onto a PD-10 column (Pharmacia) that had been equilibrated with the same buffer. The first yellow band that came out of the column was collected and the solution was concentrated with a YM-30 centricon (Amicon). The labelled protein was further purified by HPLC equipped with a size-exclusion column (Waters, Protein Pak, 8.0 × 300 mm). The mobile phase was 50 mM Tris-HCl pH 7.4 at a flow rate of 0.75 ml min^{-1}. The retention times of the labelled protein and free labels were *ca.* 10.8 and 19.9 min, respectively.

Physical measurements and instrumentation

^{1}H NMR spectra were recorded on a Varian Mercury 300 MHz NMR spectrometer at 298 K. Positive-ion ESI mass spectra were recorded on a Perkin Elmer Sciex API 365 mass spectrometer. IR spectra were recorded on a Perkin Elmer 1600 series FT-IR spectrophotometer. Elemental analyses were carried out on an Elementar Analysensysteme GmbH Vario EL elemental analyser. Electronic absorption and steady-state emission/excitation spectra were recorded on a Hewlett–Packard 8452A diode array spectrophotometer and a Spex Fluorolog-2 Model F 111 fluorescence spectrophotometer, respectively. Unless specified otherwise, all solutions for photophysical studies were degassed with no fewer than four successive freeze-pump-thaw cycles and stored in a 10 cm^3 round bottomed flask equipped with a side-arm 1 cm fluorescence cuvette and sealed from the atmosphere by a Rotaflo HP6/6 quick-release Teflon stopper. Luminescence quantum yields were measured by the optical dilute method[32] using an aerated aqueous solution of $[Ru(bpy)_3]Cl_2$ ($\Phi = 0.028$)[33] as the

standard solution. The excitation source for emission lifetime measurements was the 355 nm output (third harmonic) of a Quanta-Ray Q-switched GCR-150-10 pulsed Nd-YAG laser. Luminescence decay signals from a Hamamatsu R928 photomultiplier tube were converted to potential changes by a 50 Ω load resistor and then recorded on a Tektronix Model TDS 620A digital oscilloscope.

Crystal structure determination

Crystal data for 4b. $[C_{24.75}H_{17}Cl_{2.50}F_9IrN_3O_{9.50}S_3]$; $M = 1056.42$, triclinic, $P\bar{1}$, $a = 14.376(3)$, $b = 15.528(3)$, $c = 18.926(4)$ Å, $\alpha = 75.08(3)$, $\beta = 69.97(3)$, $\gamma = 65.96(3)°$, $\hat{U} = 3590.6(12)$ Å^3, $Z = 4$, μ(Mo-Kα) = 4.180 mm^{-1}. A crystal of dimensions 0.5 × 0.3 × 0.15 mm mounted in a glass capillary was used for data collection at 28 °C on a MAR diffractometer with a 300 mm image plate detector using graphite monochromated Mo-Kα radiation ($\lambda = 0.71073$ Å). Data collection was made with a 2° oscillation step of φ, 300 s exposure time and scanner distance at 120 mm. One hundred images were collected. The images were interpreted and intensities integrated using the program DENZO.[34] The structure was solved by direct methods (SIR-97).[35] Almost all atoms were located according to the direct methods and the successive least-squares Fourier cycles. The positions of the other non-hydrogen atoms were found after successful refinement by full-matrix least-squares refinement (SHELXL -97).[36] One water molecule was located; one CH_2Cl_2 solvent molecule was also located. Another position was found to be occupied by another CH_2Cl_2 solvent molecule. However, due to high thermal parameters of the atoms, the occupancies were set to half (a free refinement of the occupancy gave rise to a similar value); meanwhile restraints were applied to assume similar C–Cl bond lengths within a range from 1.68 to 1.72 Å. Two Ir complex molecules were located in one asymmetric unit and each contains one disordered $CF_3SO_3^-$. One molecule has F atoms disordered as rotated along the S–C bond; the other molecule has disordered S (S5 and S5′), O (O15 and O15′) and F (F13, F13′, F14, F14′, F15 and F15′) atoms. For convergence of refinements, S5′–O15′ was assumed to be near 1.38(2) Å and C(47)–F bonds were assumed to be similar. All 12465 independent reflections $\{R_{int} = 0.0368$ $(R_{int} = \sum|F_o^2 - F_o^2(\text{mean})|/\sum[F_o^2])$, 9210 reflections larger than $4\sigma(F_o)\}$ from a total 26 033 reflections participated in the full-matrix least-squares refinement against F^2. One crystallographic asymmetric unit consists of two formula units, including one water molecule, and one and a half CH_2Cl_2 solvent molecules. In the final stage of least-squares refinement, the disordered atoms and atoms of the second CH_2Cl_2 molecule (half occupancy) were refined isotropically, the other non-H atoms were refined anisotropically. H atoms were generated by the program SHELXL -97. The positions of H atoms were calculated based on a riding mode with thermal parameters equal to 1.2 times that of the associated C atoms, and participated in the calculation of final R indices. Since the structure refinements are against F^2, R indices based on F^2 are larger than (more than double) those based on F. For comparison with older refinements based on F and an OMIT threshold, a conventional index R_1 based on observed F values larger than $4\sigma(F_o)$ is also given [corresponding to intensity $\geq 2\sigma(I)$]: $wR_2 = \{\sum[w(F_o^2 - F_c^2)^2]/\sum[w(F_o^2)^2]\}^{1/2}$, $R_1 = \sum||F_o| - |F_c||/\sum|F_o|$; $w = 1/[\sigma^2(F_o^2) + (aP)^2 + bP]$, where P is $[2F_c^2 + \text{Max}(F_o^2, 0)]/3$. Convergence $[(\Delta/\sigma)_{max} = -0.001$, ave. 0.001) for 926 variable parameters by full-matrix least-squares refinement on F^2 was reached at $R_1 = 0.0473$ and $wR_2 = 0.1337$ with the parameters a and b being 0.0995 and 0.0, respectively.

CCDC reference number 175721. See http://www.rsc.org/suppdata/nj/b1/b107163g for crystallographic data in CIF or other electronic format.

Results and discussion

Synthesis

Owing to the chemical inertness of the coordination sphere of iridium(III), harsh reaction conditions are usually required for coordination of polypyridine and cyclometallating ligands. In this work, all four trichloroiridium(III) terpyridine complexes [Ir(tpy-R)Cl_3] (R = H, C_6H_5, C_6H_4-CH_3-*p*, C_6H_4-Cl-*p*) were synthesised from reactions of $IrCl_3 \cdot 3H_2O$ and the corresponding terpyridines in ethylene glycol at 160 °C for 20 min.[19b,27] The amine-containing precursor complexes [Ir(tpy-R)(tpy-C_6H_4-NH_2-*p*)]$(PF_6)_3$ [R = H (**1a**), C_6H_5 (**2a**), C_6H_4-CH_3-*p* (**3a**), C_6H_4-Cl-*p* (**4a**)] were, however, prepared by two different methods. Complex **1a** was obtained, in a moderate yield, from the reaction of [Ir(tpy-H)Cl_3] and tpy-C_6H_4-NH_2-*p* in ethylene glycol at 160 °C for 20 min, followed by metathesis with NH_4PF_6. However, we found that direct reactions of [Ir(tpy-R)Cl_3] (R = C_6H_5, C_6H_4-CH_3-*p*, C_6H_4-Cl-*p*) and tpy-C_6H_4-NH_2-*p* under similar conditions gave many side-products and the desired complexes were isolated in very low yields. Therefore, an alternative synthetic procedure for the amine-containing complexes **2a**–**4a** was sought. In view of the fact that $[Ir(bpy)_3]^{2+}$, *cis*-$[Ir(bpy)_2(PPh_3)H]^{2+}$ and *cis*-$[Ir(bpy)_2H_2]^+$ can be conveniently synthesised from the precursor complex *cis*-$[Ir(bpy)_2(CF_3SO_3)_2](CF_3SO_3)$,[37] we attempted a similar synthesis and successfully isolated a series of new intermediate complexes [Ir(tpy-R)$(CF_3SO_3)_3$], [R = C_6H_5 (**2b**), C_6H_4-CH_3-*p* (**3b**), C_6H_4-Cl-*p* (**4b**)] from the reactions of [Ir(tpy-R)Cl_3] (R = C_6H_5, C_6H_4-CH_3-*p*, C_6H_4-Cl-*p*) and trifluoromethanesulfonic acid in refluxing 1,2-dichlorobenzene. The amine-containing complexes **2a**–**4a** were then obtained by heating a mixture of **2b**–**4b** and the ligand tpy-C_6H_4-NH_2-*p* in ethylene glycol at 160 °C for 20 min, followed by anion exchange, column purification and recrystallisation from acetone–diethyl ether. We found that using [Ir(tpy-R)$(CF_3SO_3)_3$] instead of [Ir(tpy-R)Cl_3] as the precursor complexes for **2a**–**4a** can give the desired products in higher yields. It appears that tris(trifluoromethanesulfonato)iridium(III) complexes of this kind are versatile starting materials for syntheses of heteroleptic iridium(III) terpyridines.

The target complexes [Ir(tpy-R)(tpy-C_6H_4-NCS-*p*)]$(PF_6)_3$ [R = H (**1**), C_6H_4-CH_3-*p* (**2**), C_6H_5 (**3**), C_6H_4-Cl-*p* (**4**)] were prepared from reactions of the amine-containing complexes **1a**–**4a** and $CSCl_2$ in the presence of $CaCO_3$ in acetone. Similar procedures have been adopted for the syntheses of ruthenium(II)[7,8a] and osmium(II)[8d] isothiocyanate complexes $[M(N\text{-}N)_2(\text{phen-5-NCS})]^{2+}$ [M = Ru(II), Os(II); N-N 2,2′-bipyridine, 1,10-phenanthroline; phen-5-NCS = 5-isothiocyanato-1,10-phenanthroline]. The conversion of the amine moieties of **1a**–**4a** to the isothiocyanate groups of **1**–**4** was associated with a downfield shift of the NMR resonance signals of two H_m protons of tpy-C_6H_4-NH_2-*p* (from *ca.* δ 7.0 in tpy-C_6H_4-NH_2-*p* to δ 7.8 in tpy-C_6H_4-NCS-*p*) and the observation of an IR absorption peak at *ca.* 2089–2095 cm^{-1}, typical of ν_{NCS} stretching. All the new complexes were characterised by ^{1}H NMR, positive-ion ESI-MS and IR, and gave satisfactory elemental analyses.

X-Ray crystal structure determination

Compared to organometallic iridium(III) systems, crystal structures of iridium(III) terpyridine complexes are very rare.[19b,25a] Single crystals of **4b** were obtained by layering a concentrated dichloromethane solution of the complex with petroleum ether. The perspective views of the two independent molecules of **4b** with the atomic numbering scheme are shown in Fig. 1. Selected bond distances and angles are listed in Table 1. As a consequence of the geometric constraints imposed by the tpy-C_6H_4-Cl-*p* ligand, the iridium(III) centre is

in a distorted octahedral geometry, with the $CF_3SO_3^-$ ligands occupying three *meridionally* arranged coordination sites as expected. The average Ir–N bond distances (*ca.* 1.938 Å for the central pyridine ring and 2.055 Å for the peripheral ones) and the N–Ir–N bite angles (80.0–81.3°) are in excellent agreement with those observed in the related complexes $[Ir(tpy\text{-}H)_2]^{3+}$ (1.978–2.058 Å, 80.0–80.3°)[19b] and [Ir{2,3,5,6-tetrakis(2-pyridyl)pyrazine}Cl_3] (1.917–2.032 Å, 80.8°).[25a] As a result of the steric hindrance between the *meta* protons of the central pyridine ring and the *ortho* protons of the phenyl ring, these two rings are twisted about the interannular C–C bonds, resulting in dihedral angles of *ca.* 16.0 and 17.8° in the two independent molecules. These values are similar to those observed in $[Ni(tpy\text{-}C_6H_5)_2]Cl_2$ (16.7 and 17.8°)[38] but noticeably smaller than those of $[Pt(tpy\text{-}C_6H_5)Cl]$ (33.4°)[39] and [Cu{4′-(*p*-1,4,7-triazacyclonon-1-ylmethylphenyl)-2,2′:6′,2″-terpyridine}-$(H_2O)_2]^{2+}$ (24.2°).[40]

Electronic absorption and emission properties

All the new iridium(III) terpyridine isothiocyanate complexes are soluble in acetonitrile and acetone, giving yellow solutions. The electronic absorption spectral data for **1**–**4** are summarised in Table 2. As examples, the absorption spectra of **1** and **2** are shown in Fig. 2. For all four complexes, the intense absorption peaks and shoulders at *ca.* 252–372 nm with extinction coefficients of the order of 10^4 dm^3 mol^{-1} cm^{-1} are assigned to intraligand (IL) transitions. Due to the lack of 4′-aryl substituents on the tpy-H ligand of **1**, the extinction coefficients of the absorption bands are smaller than those of **2**–**4** (Fig. 2). On the other hand, all the complexes show weaker absorptions tailing into the lower energy region. It is likely that these absorption features are partially due to spin-allowed and spin-forbidden metal-to-ligand charge-transfer (MLCT) [dπ(Ir) → π*(terpyridines)] transitions. The possible observation of the latter is a result of the heavy atom effect.[41]

Upon photoexcitation, complexes **1**–**4** exhibit intense and long-lived yellow emission in CH_3CN at 298 K and green emission in low-temperature alcohol glass. The photophysical data are listed in Table 3. As an example, the emission and excitation spectra of **3** in CH_3CN at 298 K and EtOH–MeOH glass at 77 K are shown in Fig. 3(a) and 3(b), respectively. The room-temperature solution emission spectra of all the complexes exhibit structured features, with an emission maximum occurring at *ca.* 527–530 nm and a shoulder at *ca.* 555–558 nm. The emission lifetimes fall on the microsecond timescale in both degassed and aerated CH_3CN solutions (Table 3). This finding, together with the large Stokes' shifts (*ca.* 0.93–1.07 eV), suggests a phosphorescence nature of the emission. In view of the structured emission solution spectra, long emission lifetimes and small radiative decay rate constants (k_r), the emission of the complexes is assigned to an ^{3}IL [π → π*(terpyridines)] emissive state, which is mixed with some ^{3}MLCT [dπ(Ir) → π*(terpyridines)] character. The involvement of the ^{3}MLCT character is supported by the blue shifts of the emission maxima in the low-temperature glass [Table 3, Fig. 3(a) and 3(b)], since a similar shift is absent for pure ^{3}IL emitters such as $[Ir(tpy\text{-}H)_2]^{3+}$.[19b]

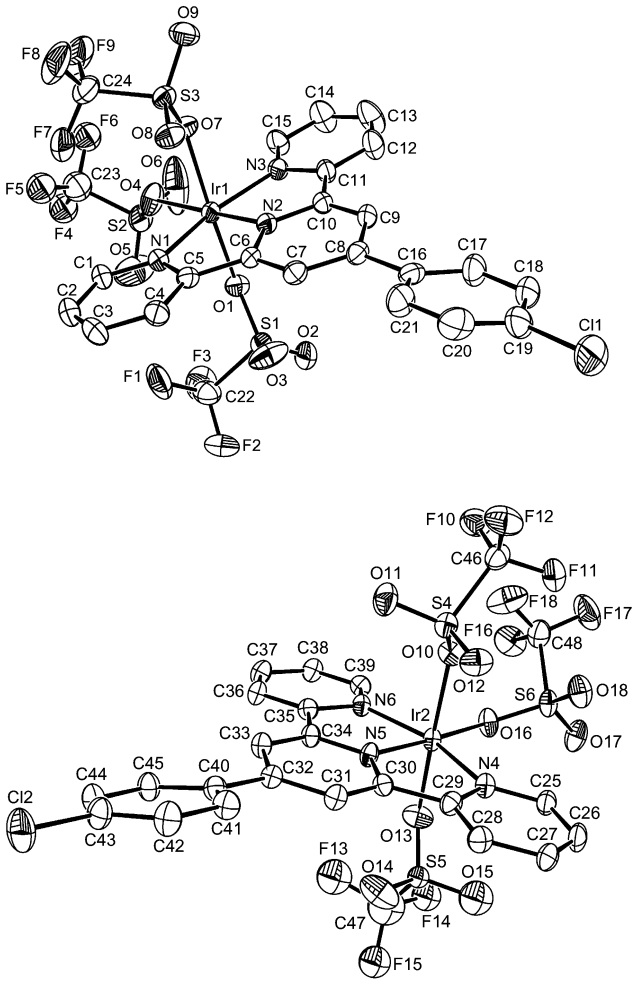


Fig. 1 Perspective drawings of the two independent molecules of **4b** with the atomic numbering scheme. Hydrogen atoms have been omitted for clarity. Thermal ellipsoids are shown at the 20% probability level.

Table 1 Selected bond distances (Å) and angles (°) for complex **4b**

Ir(1)–N(1)	2.056(7)	Ir(2)–N(4)	2.070(6)
Ir(1)–N(2)	1.938(6)	Ir(2)–N(5)	1.938(6)
Ir(1)–N(3)	2.052(7)	Ir(2)–N(6)	2.041(6)
Ir(1)–O(1)	2.055(6)	Ir(2)–O(10)	2.048(5)
Ir(1)–O(4)	2.094(6)	Ir(2)–O(13)	2.039(6)
Ir(1)–O(7)	2.059(6)	Ir(2)–O(16)	2.086(5)
N(1)–Ir(1)–N(2)	80.2(3)	N(4)–Ir(2)–N(5)	80.0(2)
N(1)–Ir(1)–N(3)	161.4(3)	N(4)–Ir(2)–N(6)	161.0(2)
N(1)–Ir(1)–O(1)	94.1(2)	N(4)–Ir(2)–O(16)	105.5(2)
N(1)–Ir(1)–O(4)	98.0(3)	N(4)–Ir(2)–O(10)	95.6(2)
N(1)–Ir(1)–O(7)	95.5(3)	N(4)–Ir(2)–O(13)	90.1(3)
N(2)–Ir(1)–N(3)	81.3(2)	N(5)–Ir(2)–N(6)	81.3(2)
N(2)–Ir(1)–O(1)	93.2(2)	N(5)–Ir(2)–O(10)	93.3(2)
N(2)–Ir(1)–O(4)	177.0(3)	N(5)–Ir(2)–O(13)	94.3(3)
N(2)–Ir(1)–O(7)	94.9(3)	N(5)–Ir(2)–O(16)	174.3(2)
N(3)–Ir(1)–O(1)	88.0(3)	N(6)–Ir(2)–O(10)	88.2(2)
N(3)–Ir(1)–O(4)	100.5(3)	N(6)–Ir(2)–O(13)	88.6(3)
N(3)–Ir(1)–O(7)	85.0(3)	N(6)–Ir(2)–O(16)	93.2(2)
O(1)–Ir(1)–O(4)	89.2(3)	O(10)–Ir(2)–O(13)	171.2(2)
O(1)–Ir(1)–O(7)	168.4(2)	O(10)–Ir(2)–O(16)	87.8(2)
O(4)–Ir(1)–O(7)	83.0(3)	O(13)–Ir(2)–O(16)	84.2(2)

Table 2 Electronic absorption spectral data for complexes **1**–**4** in CH_3CN at 298 K

	λ_{abs}/nm (ε/dm^3 mol^{-1} cm^{-1})
1	252 (44,245), 280 (41,950), 312 (29,640), 322 (29,835), 338 sh (24,660), 352 (24,140), 372 (18,835)
2	252 (48,915), 282 (47,415), 300 sh (43,615), 320 sh (38,725), 344 sh (29,980), 370 sh (23,740)
3	252 (47,665), 280 (45,295), 308 (40,620), 318 sh (39,295), 344 sh (30,625), 370 sh (25,270)
4	252 (46,450), 282 (43,190), 302 sh (40,885), 322 sh (37,300), 344 sh (30,335), 370 sh (22,960)

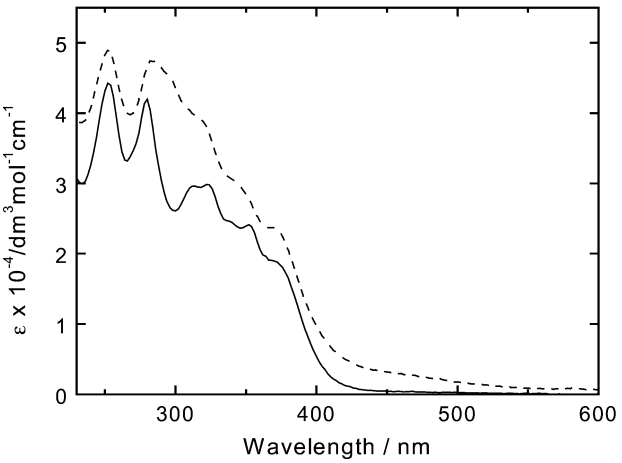


Fig. 2 Electronic absorption spectra of **1** (——) and **2** (------) in CH_3CN at 298 K.

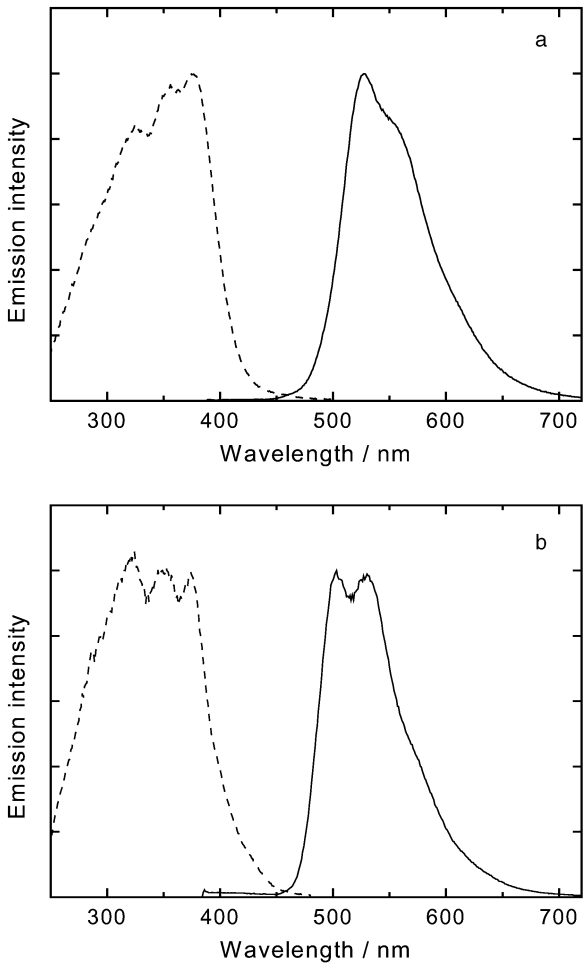


Fig. 3 Emission (——) and excitation (------) spectra of **3** in (a) CH_3CN at 298 K and (b) EtOH–MeOH (4 : 1 v/v) at 77 K.

It is likely that the tpy-C_6H_4-NCS-*p* ligand plays an important role in the ^{3}IL/^{3}MLCT states of **1** and probably in **2**–**4** as well, based on the high similarity in emission energy among the complexes (especially between **1** and **2**–**4**), although the involvement of the "ancillary" terpyridine ligands tpy-R (R = C_6H_5, C_6H_4-CH_3-*p*, C_6H_4-Cl-*p*) cannot be totally neglected. On the other hand, the emission band shapes of the complexes, as well as the long emission lifetimes, resemble those of a class of related homoleptic and heteroleptic iridium(III) terpyridine complexes, $[Ir(tpy\text{-}C_6H_3\text{-}^tBu_2\text{-}m)_2]^{3+}$, $[Ir(tpy\text{-}C_6H_4\text{-}CH_3\text{-}p)_2]^{3+}$ and $[Ir(tpy\text{-}CH_3)(tpy\text{-}C_6H_4\text{-}CH_3\text{-}p)]^{3+}$,[19b] for which a mixed ^{3}IL/^{3}MLCT excited state has been suggested, except that the emission maxima for the latter complexes occur at higher energy (*ca.* 506 nm in CH_3CN at room temperature). The lower emission energy of the complexes in the current work is attributable to a higher degree of π-conjugation in the tpy-C_6H_4-NCS-*p* ligand and the electron-withdrawing effects of the isothiocyanate group, which are both expected to stabilise the ^{3}IL/^{3}MLCT states.

The emission lifetimes of the complexes in CH_3CN at 298 K are remarkably long (Table 3). The reason for the observation that the excited state of **3** is the longest-lived (16.6 μs) while that of **4** the shortest (8.4 μs) is not fully understood. One possible explanation, however, is that the degree of ^{3}MLCT character of the excited state of **3** is lower than that of **4**. Mixing of ^{3}MLCT character into the ^{3}IL excited states of Ir(III) terpyridine complexes has been shown to shorten the emission lifetimes and lower the luminescence quantum yields considerably.[27] On the other hand, it is interesting to note that while the 77 K emission lifetime of **1** follows a single exponential (*ca.* 144 μs), **2**–**4** display double-exponential decays, with longer- and shorter-lived components of *ca.* 123–109 μs and 25–19 μs, respectively. We assign the longer-lived component, which is approximately an order of magnitude longer than the solution emission lifetimes at room temperature, to a mixed ^{3}IL/^{3}MLCT excited state involving mainly the tpy-C_6H_4-NCS-*p* ligand. The shorter-lived component appears to originate from an un-equilibrated ^{3}IL/^{3}MLCT excited state associated essentially with the "ancillary" terpyridine ligands. This assignment is based on the finding that $[Ir(tpy\text{-}C_6H_4\text{-}CH_3\text{-}p)_2]^{3+}$, a structural analogue of **3**, emits at 494 nm with a lifetime of 39 μs in 77 K glass.[19b] The absence of a shorter-lived component for **1** is probably a result of the higher energy of the ^{3}IL and ^{3}MLCT states involving the tpy-H ligand, given that the 77 K glass emission of $[Ir(tpy\text{-}H)_2]^{3+}$ occurs at 458 nm with a lifetime of 26 μs.[19b] It is noteworthy that dual luminescence decays have been reported in other heteroleptic iridium(III) terpyridine complexes in fluid solutions at room temperature.[27b]

Labelling of proteins

Since the isothiocyanate moiety can react with primary amine groups of biomolecules to form stable thiourea,[28,29] it is our intention to incorporate an isothiocyanate moiety into

Table 3 Photophysical data for complexes **1**–**4**

	$\lambda_{em}{}^a$/nm	$\tau_o{}^a$/μs	Φ^a	$k_r{}^a$/s^{-1}	$k_{nr}{}^a$/s^{-1}	τ^b/μs	$\lambda_{em}{}^c$/nm	$\tau_o{}^c$/μs
1	530, 555 sh	13.2	0.025	1.9×10^3	7.4×10^4	1.4	500, 534, 578 sh	144.0
2	528, 555 sh	12.6	0.031	2.5×10^3	7.7×10^4	1.3	500, 534, 578 sh	123.4 (74%), 24.2 (26%)
3	527, 558 sh	16.6	0.064	3.9×10^3	5.6×10^4	1.5	504, 530, 576 sh	108.9 (28%), 25.3 (72%)
4	528, 558 sh	8.4	0.030	3.6×10^3	1.2×10^5	1.4	502, 534, 578 sh	117.3 (48%), 18.8 (52%)

[a] In degassed CH_3CN at 298 K. [b] In aerated CH_3CN at 298 K. [c] In EtOH–MeOH (4 : 1 v/v) at 77 K.

Table 4 Photophysical data for conjugates **1**-HSA and **1**-BSA in 50 mM Tris-HCl pH 7.4 at 298 K

Conjugate	$\lambda_{em}{}^{a}$/nm	$\tau_o{}^{a}$/μs	τ^{b}/μs
1-HSA	530	1.52 (10%) 0.17 (90%)	1.32 (16%) 0.21 (84%)
1-BSA	530	0.99 (19%) 0.11 (81%)	0.85 (23%) 0.15 (77%)

[a] In degassed buffer. [b] In aerated buffer.

luminescent iridium(III) terpyridine complexes so that they can be used as covalent labels for biological substrates. In this work, the proteins HSA and BSA have both been labelled with complex **1**. The bioconjugates **1**-HSA and **1**-BSA were purified by size-exclusion chromatography to remove the unreacted complex. The electronic absorption spectra of the conjugates display intense absorptions in the visible region, ascribed to the absorption properties of the iridium(III) chromophores of the labels. The iridium : protein ratios have been estimated based on the absorption spectral data according to the following equation:

$$\frac{[\mathrm{Ir}]}{[\mathrm{protein}]} = \frac{A_{380}\varepsilon_{280\mathrm{p}}}{A_{280}\varepsilon_{380\mathrm{i}} - A_{380}\varepsilon_{280\mathrm{i}}}$$

where A_{380} and A_{280} are the absorbance values of the bioconjugates at 380 and 280 nm, respectively; $\varepsilon_{280\mathrm{p}}$ is the extinction coefficient of the protein at 280 nm; $\varepsilon_{380\mathrm{i}}$ and $\varepsilon_{280\mathrm{i}}$ are those of the iridium complex at 380 and 280 nm, respectively. Iridium : protein ratios of 2.7 and 1.4 have been determined for **1**-HSA and **1**-BSA, respectively. These values are similar to related systems with other transition metal complexes as labels.[7,8b,d,e,14]

Upon photoexcitation, these conjugates exhibit intense and long-lived yellow emission in 50 mM Tris-Cl buffer pH 7.4 (Table 4). The emission maxima are indistinguishable from that of the free complex, suggestive of an ^{3}IL/^{3}MLCT emissive state. This assignment is also in line with the relatively long emission lifetimes of the conjugates in aqueous buffer. Dual luminescence decays, with average longer and shorter components of *ca.* 1.3 and 0.14 μs, are observed for **1**-HSA and **1**-BSA. This reflects the difference of the local environments of the labels attached to the protein molecules. Actually, dual and multiexponential decays for biomolecules labelled with luminescent compounds have been observed in other systems.[6a,7,8,12c] In aerated Tris-Cl buffer, quenching of the emission of **1**-HSA and **1**-BSA by oxygen molecules occurs as expected, owing to the triplet character of the excited state of the label. However, it is interesting to note that emission quenching of the labelled proteins by oxygen (**1**-HSA, $k_q = 2.9 \times 10^8$ dm^3 mol^{-1} s^{-1}; **1**-BSA, $k_q = 1.3 \times 10^9$ dm^3 mol^{-1} s^{-1}) is not efficient. It is likely that the labels attached to the biomolecules are shielded in the interior of the protein amino acid residues. As a consequence, a lower exposure to the buffer environment could account for the inefficient oxygen quenching.[7,8] Nevertheless, in view of the relatively long emission lifetimes of the labelled proteins in aerated aqueous buffer, it is anticipated that the iridium(III) isothiocyanate complexes could be utilised in various time-resolved bioassays.

Summary

We have synthesised and characterised a series of new iridium(III) terpyridine complexes with an isothiocyanate functional group. The syntheses of these complexes have involved the isolation of another new series of intermediate complexes, [Ir(tpy-R)(CF$_3$SO$_3$)$_3$]. The X-ray crystal structure of one of these intermediates, **4b**, has been studied. The isothiocyanate complexes display intense and long-lived emission in degassed and aerated acetonitrile solutions at 298 K and in low-temperature glass. The emission is assigned to originate from a predominant ^{3}IL [$\pi \rightarrow \pi^*$(terpyridines)] excited state mixed with some ^{3}MLCT [dπ(Ir) $\rightarrow \pi^*$(terpyridines)] character. Two proteins, HSA and BSA, have been labelled with **1**. The bioconjugates exhibit intense and long-lived emission in aqueous buffers under ambient conditions. The potential use of these luminescent iridium(III) labelling reagents in different bioanalytical applications is currently under investigation.

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